

Acquisition of Host Cell DNA Sequences by Baculoviruses: Relationship Between Host DNA Insertions and FP Mutants of *Autographa californica* and *Galleria mellonella* Nuclear Polyhedrosis Viruses

M. J. FRASER, GALE E. SMITH, AND MAX D. SUMMERS*

Department of Entomology, Texas A&M University and Texas Agricultural Experiment Station, College Station, Texas 77843

Received 14 February 1983/Accepted 2 May 1983

Mutants of *Autographa californica* and *Galleria mellonella* nuclear polyhedrosis viruses, which produce an altered plaque phenotype as a result of reduced numbers of viral occlusions in infected cells, were isolated after passage in *Trichoplusia ni* (TN-368) cells. These mutants, termed FP (few-polyhedra) mutants, had acquired cell DNA sequences ranging from 0.8 to 2.8 kilobase pairs in size. The insertions of cell DNA occurred in a specific region between 35.0 and 37.7 map units of the *A. californica* viral genome. A cloned viral fragment containing one of the host DNA inserts was homologous to host DNA inserts in two other mutant viruses and to dispersed, repetitious sequences in *T. ni* cell DNA. Most of the homology between the cloned insert and cell DNA was contained within a 1,280-base-pair *AluI* fragment. Marker rescue studies and analysis of infected-cell-specific proteins suggested that the insertion of cell DNA into the viral genomes resulted in the FP plaque phenotype, possibly through the inactivation of a 25,000-molecular-weight protein.

Nuclear polyhedrosis viruses (NPV) produce two infectious forms: viral occlusions (polyhedra) and nonoccluded, extracellular virus. The viral occlusions are proteinaceous crystals in which many virions are embedded. They are assembled in the nuclei of infected cells and are responsible for the horizontal transmission of the disease. Nonoccluded, extracellular virus (ECV) acquire envelopes by budding through the plasma membrane of infected cells and are responsible for secondary infection within an infected insect or cultured cells.

Serial propagation of *Autographa californica*, *Trichoplusia ni*, and *Galleria mellonella* MNPVs (14, 18, 31) and *Heliothis zea* SNPV (M. J. Fraser and W. J. McCarthy, unpublished data) in cell cultures with the ECV as inoculum results in a progressive increase in the percentage of a class of spontaneous mutants which produce fewer occlusions in infected cells than the wild-type virus. Most of the mutant occlusions are devoid of nucleocapsids, whereas some contain fewer nucleocapsids or defective particles (14, 18, 31). These mutants, termed FP (few-polyhedra) mutants, are easily detected because they produce a distinctive plaque morphology (13, 18, 31). FP mutants are usually detected within three or four passages in cultured cells (14, 17), are nondefective for replication (14, 31), and

have been isolated from infected insects and cultured cells (14, 17). These features distinguish FP mutants from mutagen-induced occlusion morphology mutants (5, 9) or temperature-sensitive mutants (4, 26), occlusion-negative mutants produced by site-directed mutagenesis (35), or the many morphogenic mutants with defective occlusions that have been observed by electron microscopy after long term (30 or more) serial passages (19, 23, 27, 33). Kelly (22) recently reviewed some of the properties of FP mutants.

Miller and Miller (28) describe a FP mutant of *A. californica* MNPV which contains an insertion of host cell DNA between 86.4 and 86.6 map units of the *A. californica* MNPV genome. They also demonstrate that this insertion is homologous to moderately repetitive host DNA and structurally resembles a *Drosophila copia*-like element. They speculate that the inserted DNA is probably responsible for generating the FP phenotype. However, they also note that other FP mutants have been described which contain no discernable insertions (31), whereas insertions are found in phenotypically wild-type virus (6). These data suggest that there may be more than one mechanism to produce the FP phenotype.

In the present study, we obtained FP mutants from plaque-purified strains of the closely relat-

ed baculoviruses, *A. californica* MNPV (AcMNPV) and *G. mellonella* MNPV (GmMNPV) (36) and demonstrated that, although FP mutants have several different genotypes, most had in common an insertion of host cell DNA in a restricted region of the viral genome. The cell DNA insert in each FP mutant genome was unique. Hybridization analysis of some of the DNA insertion sequences demonstrated that they were homologous to moderately repetitive host DNA. Significant homology and restriction site similarities were observed between three of eight cellular DNA insertions. The results of marker rescue studies indicated that host DNA insertions in the FP genomes may be related to the FP phenotype. An analysis of infected-cell-specific proteins demonstrated that an early viral gene encoding a 25,000-molecular-weight protein was not expressed by FP mutants.

MATERIALS AND METHODS

Preparation of virus and isolation of clones. *G. mellonella* MNPV was originally obtained from G. R. Stairs as frozen infected larvae. Viral occlusions were isolated from homogenates of infected larvae as described by Fraser and Hink (14). The E2 strain of *A. californica* MNPV (36) was propagated in *T. ni* larvae, and viral occlusions were isolated as above.

Preparation of ECV from infected *G. mellonella* or *T. ni* larvae and inoculation of the *T. ni* TN-368 (16) cell cultures were as described previously (14). Plaques which exhibited the MP (many polyhedra, wild-type) morphology in 0.75% agarose overlay assays (12) of first cell-culture-passaged virus were purified (14). These isolates were used as inocula for subsequent passages at multiplicities of 1 to 5 PFU per cell.

Each plaque-purified MP isolate was passed individually through three successive cultures of *T. ni* cells and assayed for the presence of FP mutant plaques. Several FP plaques from each MP passage series were plaque-purified as described above. These were used as inocula for 100-ml suspension cultures.

Spodoptera frugiperda IPLB-SF21AE cells (42) were transfected with AcMNPV DNA which was isolated from occlusion bodies derived from infected *T. ni* larvae. One FP mutant was plaque-purified, designated AcFP6, and was grown in *S. frugiperda* cells.

Suspension cultures and isolation of ECV DNA. Suspension cultures of *T. ni* or *S. frugiperda* cells (14) were inoculated at densities of 1.5×10^6 (*T. ni*) or 3.0×10^6 (*S. frugiperda*) cells per ml with about 0.5×10^7 to 1.0×10^7 PFU of virus. After 2 to 3 days at 29°C, viral occlusions and cell debris were removed by centrifugation at $7,500 \times g$ for 20 min.

The ECV was pelleted from the clarified cell culture supernatants by centrifuging at $100,000 \times g$ for 30 min. The virus pellets were suspended in 10 ml of TEB (TEB is 0.01 M Tris-hydrochloride–0.001 M EDTA, pH 7.5), and the virus was purified by banding in linear 25 to 50% (wt/wt) sucrose gradients (made in TEB) at $100,000 \times g$ for 1 h. The virus bands were collected and diluted with three volumes of TEB, and the virus

was pelleted by centrifugation at $100,000 \times g$ for 30 min. The virus pellets were suspended in 1 ml of 0.15 M KCl–0.01 M Tris-hydrochloride–0.01 M EDTA (pH 7.5), heated to 65°C for 15 min, and then cooled to 55°C. The proteins were digested for 1 h at 55°C by adding sodium dodecyl sulfate (SDS) to 1% and proteinase K to 0.1 mg/ml. The proteinase K-treated virus preparations were extracted once with equal volumes of buffer-saturated phenol and chloroform–isoamyl alcohol (24:1), followed by two extractions with two volumes of chloroform–isoamyl alcohol (24:1). The extracted preparations were dialyzed extensively against $0.1 \times \text{SSC}$ ($1 \times \text{SSC}$ is 0.015 M sodium citrate–0.15 M NaCl, pH 7.5).

Restriction enzyme analyses and Southern transfer. Plasmid or viral DNAs were digested with restriction endonucleases *AluI*, *EcoRI*, *HindIII*, *ClaI*, *SacI*, *Sall*, *PstI*, *XhoI*, *XbaI*, or *BamHI* under conditions specified by the manufacturer (Bethesda Research Labs). The digested DNAs were fractionated on horizontal 0.75% agarose gels (20 by 20 cm) (36). After electrophoresis, the gels were soaked with gentle agitation in two changes of 0.25 M HCl for 15 min each, followed by a brief rinse in distilled water and two 15-min washes in 0.5 M NaOH–1.5 M NaCl. The gels were neutralized with two 30-min washings in 1 M NH_4 acetate–0.02 M NaOH and transferred to nitrocellulose sheets (presoaked in the same buffer) by the bidirectional modification (37) of the Southern transfer technique (40).

Isolation of nuclear DNAs. Nuclear DNA was isolated from a total of 1.5×10^8 to 2.0×10^8 *T. ni* or *S. frugiperda* cells. The cells were pelleted at $300 \times g$ for 15 min, suspended in 10 ml of 30 mM Tris-hydrochloride–10 mM KCl–2 mM magnesium acetate–1% Nonidet P-40 (pH 7.4), and vortexed vigorously at 10-min intervals. Cell lysis was monitored by microscopic examination. The nuclei were pelleted from the lysed cell preparation by centrifugation at $2,000 \times g$ for 5 min, suspended in 15 ml of TEB containing 0.15 M KCl, and heated at 65°C for 15 min. Proteins were digested by adjusting the preparation to final concentrations of 1% SDS and 0.1 mg of proteinase K per ml and heating at 55°C for 3 h. The DNA was purified by extracting with phenol and chloroform–isoamyl alcohol as described above and dialyzed extensively against $0.1 \times \text{SSC}$.

Host cell DNA was cleaved with 5 units of restriction endonuclease per μg of DNA at 37°C for 8 to 12 h; 5 μg per well was loaded onto 0.75% agarose gels and electrophoresed as described above. The DNA fragments were transferred unidirectionally to nitrocellulose filters (44).

Electroelution of DNA fragments. *HindIII* restriction fragments were isolated from agarose gels after electrophoresis of 25 to 30 μg of digested viral DNA. The UV-visible fragments desired were cut from the gels, and the DNA was electroeluted and concentrated in 10 mM Tris-hydrochloride–5 mM glycine–0.1% SDS (pH 8.3) by the method of Allington et al. (1) as described by Smith and Summers (39).

Cloning of viral DNA fragments. Cloning was carried out with the pUC8 plasmid and *Escherichia coli* JM83, both supplied by Bethesda Research Labs. The procedures for ligation, transformation, amplification, and isolation of the plasmids were essentially as outlined by Smith et al. (39).

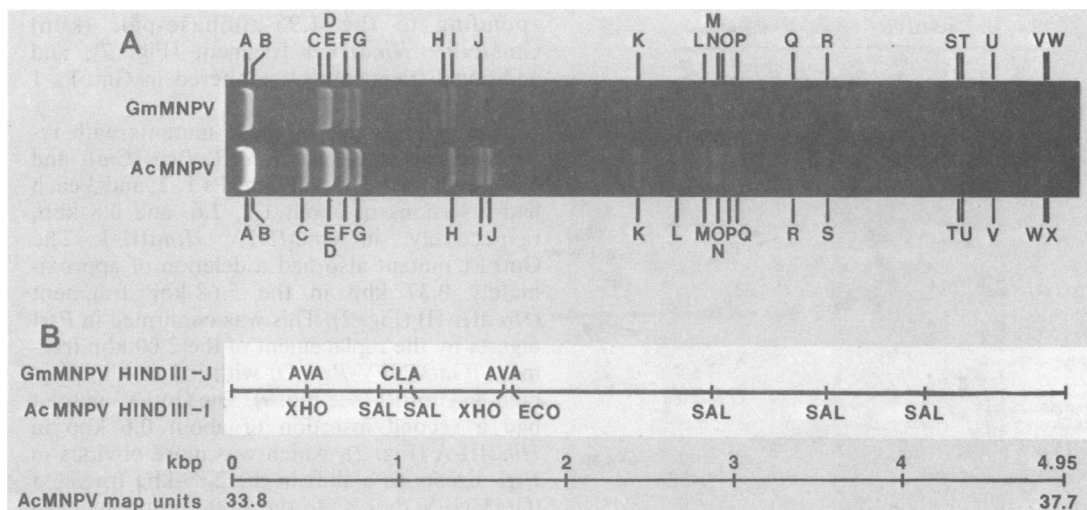


FIG. 1. Restriction enzyme site map of GmMNPV *HindIII*-J and AcMNPV *HindIII*-I. (A) *HindIII* digests of AcMNPV E2 and GmMNPV C3 DNAs were electrophoresed in 0.75% agarose gels and stained with ethidium bromide. (B) The locations of *AvaI* (AVA), *ClaI* (CLA), *EcoRI* (ECO), *SalI* (SAL), and *XhoI* (XHO) restriction enzyme sites were mapped in AcMNPV *HindIII*-I and GmMNPV *HindIII*-J. The size in kbp and the approximate location of this fragment on the AcMNPV E2 genome are shown.

Nick-translation and hybridization. DNAs were nick-translated with [α - 32 P]deoxycytidine 5'-triphosphate (New England Nuclear Corp.) by the method of Rigby et al. (34). Nitrocellulose blots of restriction enzyme gels were prepared for hybridization as previously described (37). Hybridization was carried out with 100,000 cpm of labeled DNA per ml for 24 h at 45°C in buffer composed of 5× SSC, 5× Denhardt solution (1× Denhardt solution is 0.02% bovine serum albumin–0.02% polyvinylpyrrolidone–0.02% Ficoll), 20 mM sodium phosphate (pH 6.5), 50% deionized formamide, and 100 μ g of sonicated, denatured calf thymus DNA (37).

Transfection of *S. frugiperda* cells with viral and plasmid DNA. Transfection of monolayers of 3.0×10^6 *S. frugiperda* cells in either plastic petri plates (60 by 15 mm) or flasks (25 cm²) was essentially as described by Burand et al. (7). Transfection with viral DNA alone was done at concentrations of 0.01 μ g of DNA per ml; plasmid DNAs were added to final concentrations of 0.1 μ g per ml for rescue experiments.

Labeling of infected-cell-specific proteins. Virus-induced proteins were identified in *S. frugiperda* cells infected with 10 PFU per cell of each virus isolate by pulse-labeling infected and mock-infected cell cultures with [3 H]leucine as described by Vlak et al. (43). Cultures were pulsed for 3-h intervals at 9, 15, and 24 h postinfection. Labeled proteins were analyzed on 10% polyacrylamide slab gels by the method of Laemmli (24) and detected by fluorography by the method of Bonner and Laskey (2).

RESULTS

Isolation of FP mutants. Each of the plaque-purified GmMNPV MP isolates from which FP mutants were later derived exhibited identical restriction enzyme digest patterns with *EcoRI*,

HindIII, *ClaI*, *BamHI*, *SalI*, *PstI*, *XhoI*, and *XbaI* (data not shown). This common genotype was designated GmMNPV strain C3 and was the predominant genotype in the original isolate. All of the plaque-purified MP isolates derived from AcMNPV strain E2 had identical patterns when analyzed with the above enzymes (data not shown).

Three FP mutants were obtained from GmMNPV (GmFPs 1 to 3) and five from AcMNPV (AcFPs 1 to 5) after three passages in *T. ni* cells at multiplicities of 1 to 5 PFU per cell. Between 0.1 and 5% of the plaques produced from each passage series were FPs by the end of the third passage. Several FP plaques were purified from each passage series, and subsequent analyses with restriction enzymes showed that only one FP genotype predominated in each. One FP mutant, AcFP6, was isolated from *S. frugiperda* cells transfected with AcMNPV as described above.

Cloning of the GmMNPV *HindIII* J and AcMNPV *HindIII* I fragments. The *HindIII* J fragment of GmMNPV and the *HindIII* I fragment of AcMNPV have similar mobilities in agarose gels (Fig. 1). Because these fragments were altered in most of the FP mutants (Fig. 2 and 3), they were cloned into the plasmid pUC8, and *EcoRI*, *ClaI*, *AvaI*, *XhoI*, and *SalI* restriction endonuclease sites were mapped.

The restriction maps of the cloned *HindIII* J fragment of GmMNPV and *HindIII* I fragment of AcMNPV were identical (Fig. 1). Because they had identical restriction maps, we selected

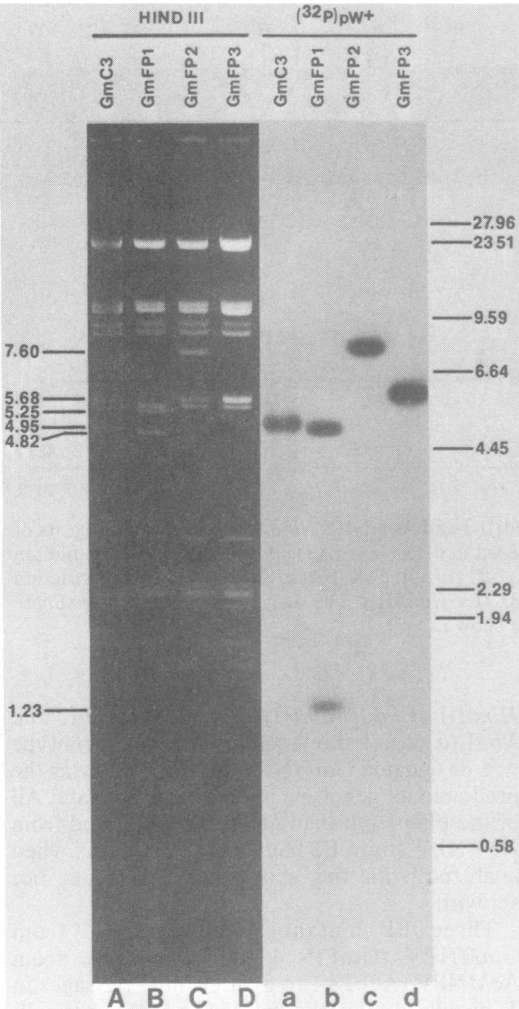


FIG. 2. Hybridization of *Hind*III restriction enzyme fragments of GmMNPV and GmFP viral DNAs with labeled GmMNPV *Hind*III-J. (Lanes A through D) Viral DNAs of GmMNPV C3 (GmC3) and GmFPs 1, 2, and 3 were digested with *Hind*III, and the fragments were electrophoresed in 0.75% agarose gels. The DNA fragments were transferred to nitrocellulose and hybridized to labeled clones of GmMNPV *Hind*III-J (pW⁺) (lanes a through d). Fragment sizes (kbp) are listed on the left, and the sizes and relative position of lambda *Hind*III fragments are indicated on the right.

the plasmid containing GmMNPV *Hind*III-J, designated pW⁺, for all hybridization experiments.

Restriction enzyme analysis of FP mutants. Three FP mutants derived from GmMNPV (FP1, FP2, and FP3) had detectable differences in restriction enzyme digest patterns when compared with those of the parent virus. All of these FP mutants were missing bands corre-

sponding to the 4.95-kilobase-pair (kbp) GmMNPV *Hind*III J fragment (Fig. 2), and additional fragments were altered in GmFPs 1 and 3.

From an analysis of these mutants with restriction endonucleases *Eco*RI, *Pst*I, *Cla*I, and *Sal*I, we concluded that GmFPs 1, 2, and 3 each had insertions of about 1.2, 2.6, and 0.8 kbp, respectively, in GmMNPV *Hind*III-J. The GmFP1 mutant also had a deletion of approximately 0.37 kbp in the 5.68-kbp fragment (*Hind*III H) (Fig. 2). This was confirmed in *Pst*I digests by the replacement of the 2.00-kbp fragment (GmMNPV-*Pst*I O) with a 1.68-kbp fragment in GmFP1 (see Fig. 9). The GmFP3 mutant had a second insertion of about 0.6 kbp in *Hind*III-A (Fig. 2), which was more obvious in *Pst*I digests as a shift in the 2.89-kbp fragment (GmMNPV *Pst*I K) to the 3.50-kbp fragment in GmFP3 (see Fig. 9).

Each FP mutant derived from AcMNPV exhibited differences in restriction enzyme digest patterns (Fig. 3). The FP mutants 1, 3, 4, 5, and 6 were each missing the *Hind*III I, *Eco*RI F, and *Pst*I E fragments of AcMNPV as a result of insertions of 1.2, 2.5, 2.5, 2.8, and 0.9 kbp, respectively, in *Hind*III-I (Fig. 3). In addition, the AcFP1 genome had a 17-kbp deletion in the 10.53-kbp fragment (AcMNPV *Hind*III D) (Fig. 3).

The AcFP2 genome had no apparent differences in *Hind*III-I (Fig. 3) but did contain an additional *Cla*I site (map location unknown) not present in the AcMNPV genome (data not shown).

Hybridization of FP mutants with labeled pW⁺. The restriction enzyme analysis suggested that there were insertions in the 4.95-kbp fragments of GmMNPV (*Hind*III-J) or AcMNPV (*Hind*III-I) in all but one of the FP mutants isolated. To confirm that these fragments had acquired additional DNA sequences, we hybridized *Hind*III-digested viral DNAs to ³²P-labeled pW⁺. The results (Fig. 2 and 3) demonstrated that in all of the FP mutants, except AcFP2, the labeled pW⁺ was homologous to either one or two new fragments which were composed of GmMNPV *Hind*III-J or AcMNPV *Hind*III-I plus inserted sequences.

Hybridization of [³²P]pW⁺ to Southern transfers of FP mutant DNAs restricted with *Hind*III, *Eco*RI, *Pst*I, and *Cla*I allowed us to construct maps of each of the FP insertions within the 4.95-kbp fragment. Restriction maps for these fragments and the approximate locations of insertion sequences are presented in Fig. 4.

Homology between *T. ni* cell DNA and the GmFP mutant genomes. *Hind*III digests of GmMNPV and GmFP viral DNAs were transferred to nitrocellulose and hybridized to ³²P-

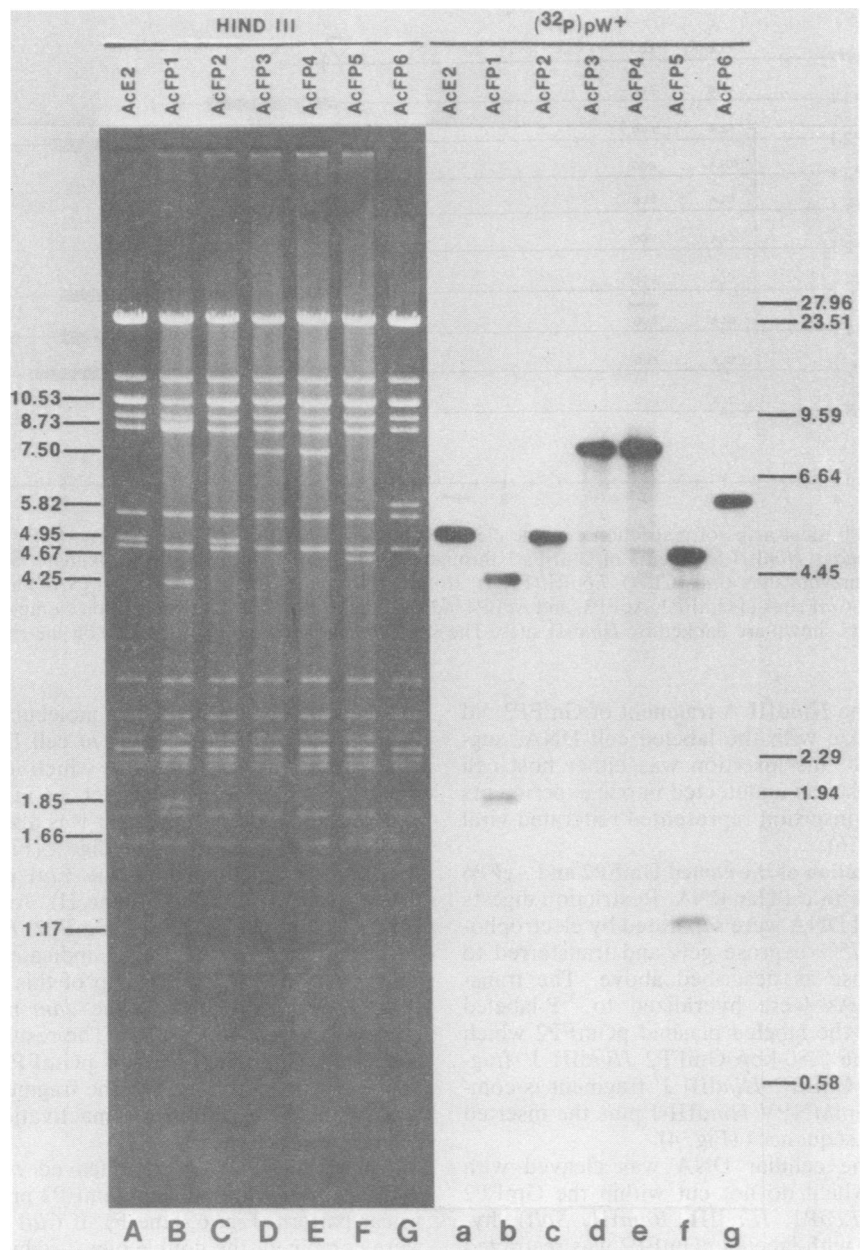


FIG. 3. Hybridization of *Hind*III (HIND III) restriction enzyme fragments of AcMNPV and AcFP viral DNAs to labeled GmMNPV *Hind*III-J. (Lanes A through G) Viral DNAs of AcMNPV E2 (AcE2) and AcFPs 1 through 6 were digested with *Hind*III and electrophoresed in 0.75% agarose gels. The fragments were transferred to nitrocellulose and hybridized with ³²P-labeled GmMNPV *Hind*III-J (pW⁺) (lanes a through g). Fragment sizes (kbp) are listed on the left. The sizes (kbp) and relative position of lambda *Hind*III fragments are indicated on the right.

labeled *T. ni* cell DNA. All of the altered fragments in the GmFP mutants which contained *Hind*III-J sequences (Fig. 3) also hybridized with the labeled cell DNA (Fig. 5). The FP1 insertion sequences hybridized most strongly with the cell DNA, indicating that these homolo-

gous sequences may have been derived from more highly repeated cellular DNA than those present in GmFPs 2 and 3. The host cell DNA in GmFP3 was apparently represented infrequently in the *T. ni* genome based on the degree of hybridization obtained. The additional DNA de-

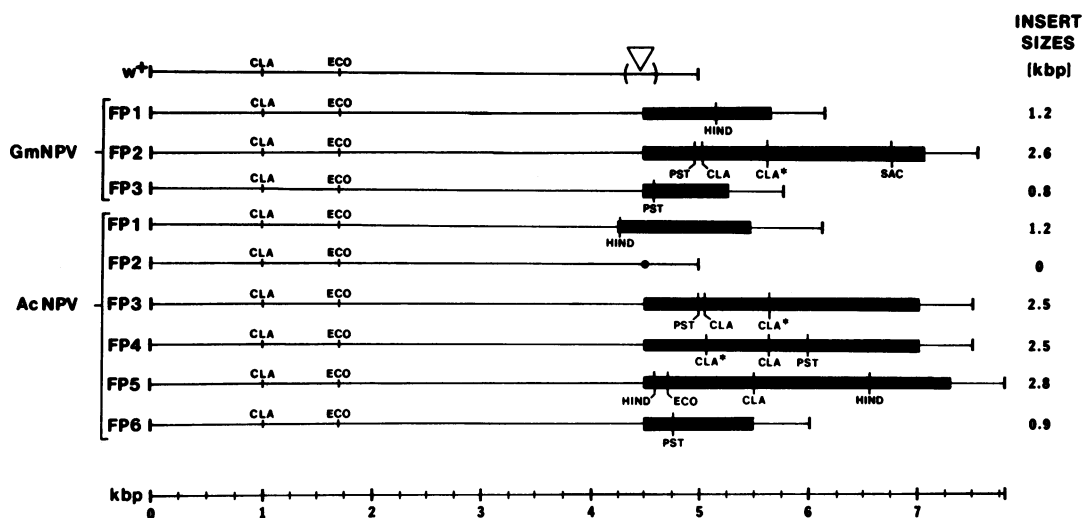


FIG. 4. Physical maps of restriction enzyme cleavage sites of GmMNPV *HindIII*-J or AcMNPV *HindIII*-I and homologous *HindIII* fragments of GmFPs 1 through 3 and AcFPs 1 through 6. Approximate locations of restriction enzyme sites *Cla*I (CLA), *Eco*RI (ECO), *Hind*III (HIND), *Pst*I (PST), and *Sac*I (SAC) are shown. Asterisks at *Cla*I sites in GmFP2, AcFP3, and AcFP4 indicate sites which are inactivated upon cloning in pUC8. All fragments shown are flanked by *Hind*III sites. The size of each insertion (kbp) is listed on the right.

tected in the *Hind*III A fragment of GmFP3 did not hybridize with the labeled cell DNA, suggesting that this insertion was either host cell DNA which was undetected in our experiments or that the insertion represented reiterated viral sequences (6).

Hybridization of the cloned GmFP2 and AcFP6 insertions with cellular DNA. Restriction digests of *T. ni* cell DNA were separated by electrophoresis in 0.75% agarose gels and transferred to nitrocellulose as described above. The transferred DNAs were hybridized to ³²P-labeled pW⁺ or to the labeled plasmid pGmFP2 which contains the 7.80-kbp GmFP2 *Hind*III J' fragment. The GmFP2 *Hind*III J' fragment is composed of GmMNPV *Hind*III-J plus the inserted host DNA sequences (Fig. 4).

When the cellular DNA was cleaved with enzymes which do not cut within the GmFP2 insertion (*Eco*RI, *Hind*III, *Bam*HI, *Sal*I), hybridization with labeled pGmFP2 was restricted to the high molecular weight regions of the gel (Fig. 6). Although smear patterns were obtained with these enzymes, some distinct bands could be distinguished above the background, such as the 12.51-kbp fragments in the *Eco*RI and *Hind*III digests, the 16.06- and 12.51-kbp fragments of the *Bam*HI digest, and the 16.06-kbp fragment of the *Sal*I digest.

When enzymes or enzyme combinations which cleaved within the insertion were employed, such as *Cla*I, *Cla*I plus *Sac*I (Fig. 6), or *Alu*I (Fig. 7), almost all of the homology between the GmFP2 insert and *T. ni* cell DNA was

reduced to one or two low molecular weight bands. The *Cla*I digest of *T. ni* cell DNA produced a 0.59-kbp fragment which contained most of the homologous sequences in pGmFP2 (Fig. 6, lane E). This fragment was also present in the *Cla*I plus *Sac*I double digest (Fig. 6, lane G) but was not present in the *Cla*I plus *Sac*I digest of pGmFP2 (Fig. 6, lane H). Analysis of several different clones of GmFP2 *Hind*III-J' suggested that the *Cla*I site, indicated by the asterisk in the restriction map of this insertion (Fig. 4), is inactivated by the *dam* methylase present in *E. coli* JM 83 (15). The resulting *Cla*I plus *Sac*I digested fragment of pGmFP2 at 1.53 kbp corresponded in size to the fragment which was predicted based on the inactivation of the designated *Cla*I site.

When cellular DNA was cleaved with *Sac*I, hybridization with labeled pGmFP2 produced a smear pattern (Fig. 6, lane F). If *Cla*I and *Sac*I were combined, the double digest reduced nearly all of the homology to two bands at 0.86 and 0.59 kbp (Fig. 6, lane G). These bands corresponded in size to *Cla*I plus *Sac*I fragments which were predicted based on the restriction map of the insertion (Fig. 4). These digests suggest that the repeated sequences represented by the 0.59-kbp *Cla*I fragment are flanked by different sequences in the *T. ni* cell genome.

After hybridization of labeled pGmFP2 with *Alu*I-digested *T. ni* cell DNA, one prominent band at 1,280 base pairs (bp) and two other bands at 430 and 410 bp, which were homologous to sequences in the GmFP2 insert, were

detected (Fig. 7). A 1,280-bp *AluI* fragment was also present in pGmFP2 which hybridized with the labeled pGmFP2 but not with labeled pW⁺ (Fig. 7) indicating that the 1,280-bp *AluI* fragment was within the insert. A 960-bp *AluI* fragment in the digest of pGmFP2 hybridized to both labeled pW⁺ and labeled pGmFP2 and mapped at the end of the insertion in GmFP2 (Fig. 7). Labeled pW⁺ had no detectable homology to cellular DNA, indicating that the hybridization observed between pGmFP2 and cell DNA was due to the inserted host sequences. These data showed that the insertion in GmFP2 contained a sequence which was homologous to a family of dispersed, repetitive DNAs in the *T. ni* cell genome. This repetitive DNA contained a 1,280-bp *AluI* fragment which was homologous to an *AluI* fragment of similar size in the GmFP2 insertion.

Hybridization of the cloned insert-containing fragment of AcFP6 (pAcFP6) to nitrocellulose blots of *T. ni* cell DNA (Fig. 8) showed that this insertion was also homologous to dispersed, repetitive DNA in the *T. ni* cell genome.

Homology between the GmFP2 insertion and other FP insertion sequences. The restriction maps of the insertions in GmFP2, AcFP3, and AcFP4 suggested that they might contain homologous sequences (Fig. 4). These three insertions contained *Clal* sites at approximately the same locations, and *Clal* digests of these mutant viral DNAs produced unique bands of 0.59-kbp (Fig. 4). Labeled pGmFP2 hybridized to the 0.59-kbp *Clal* fragments in AcFP3 and AcFP4, demonstrating that these three insertions contained homologous cell sequences (data not shown).

The plasmid clone containing the 5.82-kbp *HindIII* I' fragment of AcFP6 (pAcFP6) was labeled and hybridized to transferred restriction fragments of *T. ni* cell DNA. The inserted sequence in AcFP6 was also homologous to repetitive *T. ni* cell DNA, but these sequences were less numerous than the GmFP2 insert sequences in the cell genome.

Marker rescue of GmFP mutants with pW⁺. Experiments were conducted with the three GmFP mutants to determine whether the insertion of host cell DNA in *HindIII*-J could be correlated with the FP phenotype by rescuing the MP phenotype. *S. frugiperda* cells were transfected with purified DNAs from GmFP mutants 1, 2, and 3, either alone or with pW⁺ plasmid DNA, and progeny virus was screened for the presence of MP plaques by plaque assay in *T. ni* cells (10).

A few MP plaques could be detected among progeny virus from cells transfected with the GmFP3 mutant viral DNA alone, whereas none were detected in progeny from GmFP1 or

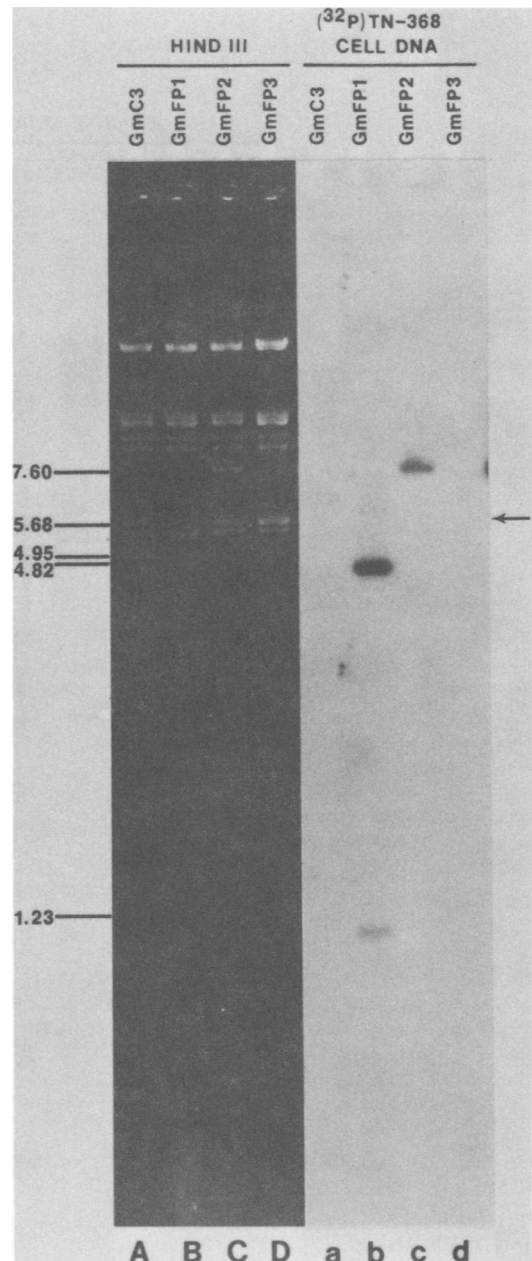


FIG. 5. Hybridization of *HindIII* (HIND III) fragments of GmMNPV and GmFP mutant viral DNAs to labeled *T. ni* cell DNA. (Lanes A through D) Viral DNAs of GmMNPV C3 (GmC3) and GmFPs 1 through 3 were digested with *HindIII* and electrophoresed in 0.75% agarose gels. The DNA fragments were transferred to nitrocellulose and hybridized to labeled *T. ni* (TN-368) cell DNA. The sizes (kbp) of certain restriction fragments are indicated on the left. The hybridization of labeled *T. ni* cell DNA to the 5.68-kbp fragment of GmFP3 (indicated by the arrow) was easily detected in the original autoradiogram.

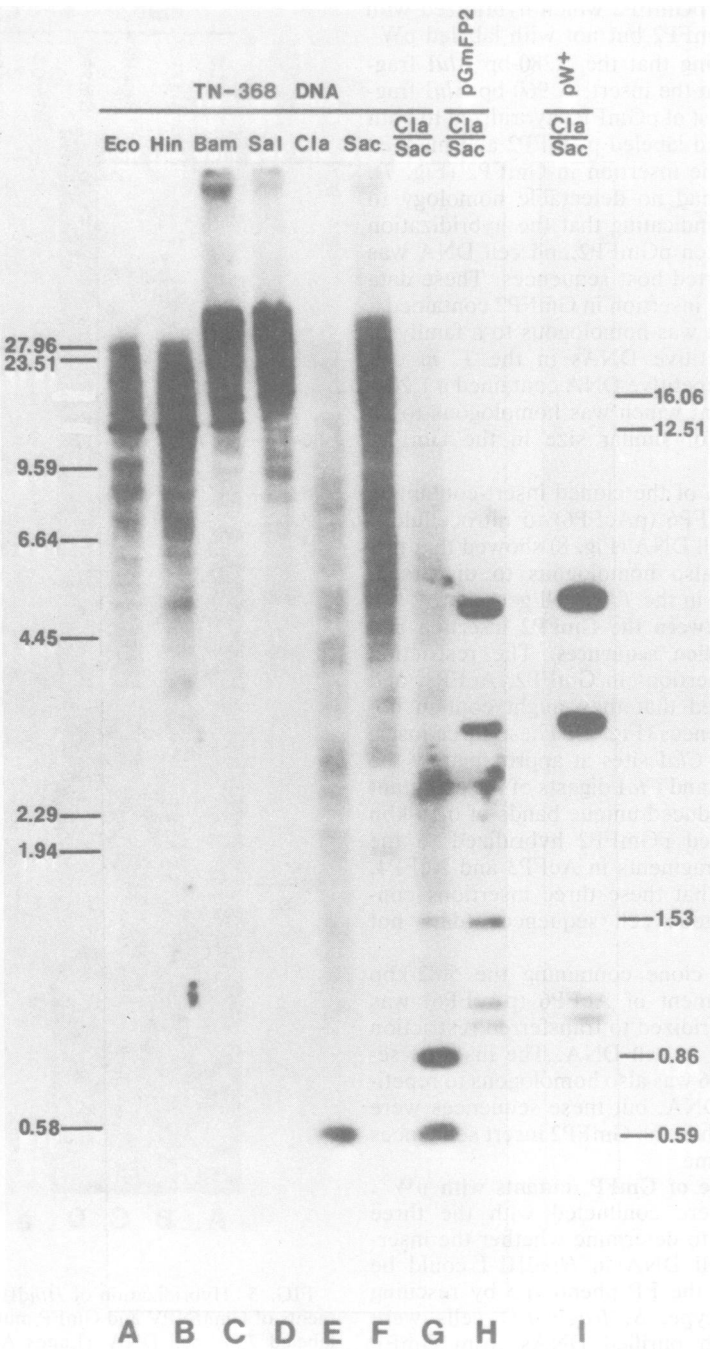


FIG. 6. Hybridization of labeled pGmFP2 plasmid DNA to restriction digests of *T. ni* (TN-368) cell DNA cleaved with *Eco*RI (Eco), *Hind*III (Hin), *Bam*HI (Bam), *Sal*I (Sal), *Cla*I (Cla), *Sac*I (Sac), or a combination of *Cla*I and *Sac*I (Cla/Sac) (lanes A through G), and Cla/Sac digests of the plasmids pGmFP2 and pW⁺ (lanes H and I) were electrophoresed in 0.75% agarose gels. The DNA fragments were transferred to nitrocellulose and hybridized to ³²P-labeled pGmFP2. Sizes (kbp) of certain fragments are indicated on the right, and the sizes and relative positions of lambda *Hind*III fragments are indicated on the left.

GmFP2 transfections (Table 1). Restriction enzyme digests of these spontaneous revertants of GmFP3 showed that they were lacking the insertion in the 4.95-kbp *Hind*III J fragment but retained the insertion in the 23.0-kbp *Hind*III A fragment. The presence of this insertion was more apparent in the 2.89-kbp *Pst*I K fragment (Fig. 9).

Progeny virus from cells cotransfected with the GmFP mutant DNAs and pW⁺ plasmid DNA exhibited significantly higher percentages of MP plaques (Table 1). No plaques were detected in assays of supernatants from cells transfected with the pW⁺ plasmid DNA alone.

Restriction enzyme digests of several rescued GmFP1 and GmFP3 viruses (GmFP1r and GmFP3r) showed that the insertions in the 4.95-kbp fragment (*Hind*III-J) or the 10.06-kbp frag-

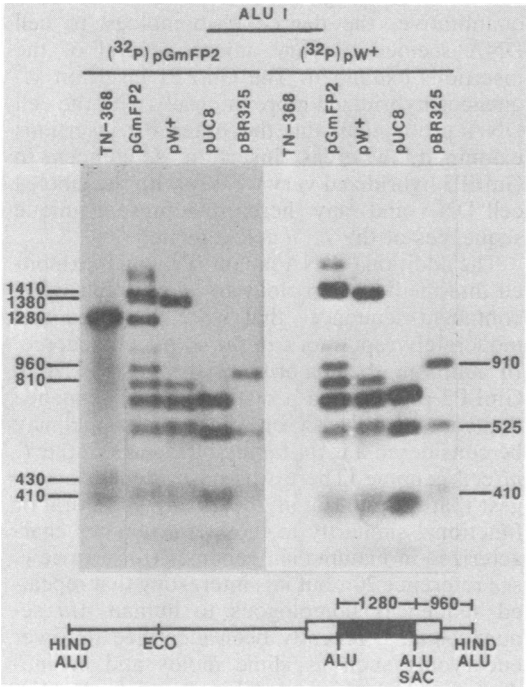


FIG. 7. Hybridization of labeled GmFP2 *Hind*III-J' and GmMNPV *Hind*III-J to *Alu*I fragments of *T. ni* cellular DNA. *T. ni* (TN-368) cell DNA and plasmids pGmFP2, pW⁺, pUC8, and pBR325 were digested with *Alu*I and electrophoresed in 1.5% agarose gels. The DNA fragments were transferred to nitrocellulose sheets and hybridized to ³²P-labeled GmFP2 *Hind*III-J' (pGmFP2) or GmMNPV *Hind*III-J (pW⁺). Size (bp) of certain *Alu*I fragments that hybridized to the labeled probes are listed on the left. The sizes (bp) of pBR325 *Alu*I fragments used as molecular weight markers are indicated on the right. A map showing the location of the 1,280- and 960-bp GmFPs *Alu*I fragments, *Hind*III (HIND), *Eco*RI (ECO), *Sac*I (SAC), and some known *Alu*I (ALU) restriction enzyme sites is presented.

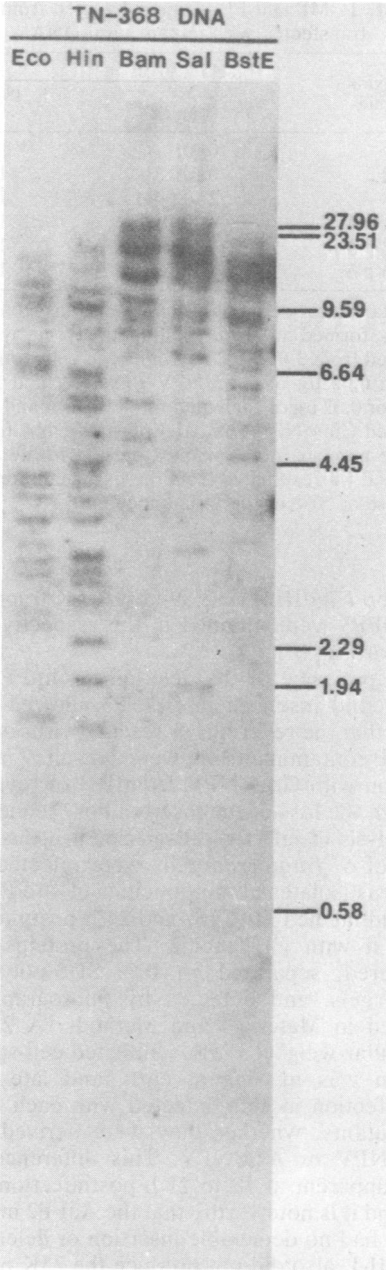


FIG. 8. Hybridization of labeled pAcFP6 to *T. ni* cell DNA. *T. ni* (TN-368) cell DNA was cleaved with *Eco*RI (Eco), *Hind*III (Hin), *Bam*HI (Bam), *Sal*I (Sal), or *Bst*EI (BstE) and electrophoresed in a 0.75% agarose gel. The fragments were transferred to nitrocellulose and hybridized to ³²P-labeled pAcFP6. The sizes (kbp) and locations of lambda *Hind*III fragments are indicated on the right.

ment (*Pst*I-E) were no longer present in any of the rescued virus clones (Fig. 9). The deletion in the 5.68-kbp *Hind*III H or 2.00-kbp *Pst*I N fragments in GmFP1 and the insertion in the

TABLE 1. MP and FP plaques detected from cells transfected with mutant viral DNA

DNA viral plasmid	No. (%) of MP plaques ^a	No. of FP plaques × 10 ^{4b}
GmFP1	0 (0)	9
GmFP2	0 (0)	13
GmFP3	2 (0.001)	16.5
GmFP1 pW ⁺	16 (0.013)	12
GmFP2 pW ⁺	12 (0.007)	16.5
GmFP3 pW ⁺	13 (0.007)	18

^a Data were derived from visual inspection of plaques formed in *T. ni* cell monolayers by virus produced from 3.0×10^6 *S. frugiperda* cells transfected with 0.02 µg of GmMNPV FPs 1, 2, and 3 viral DNAs or 0.02 µg of each mutant viral DNA and 0.2 µg of cloned GmMNPV HindIII-J plasmid DNA (pW⁺).

^b The number of FP plaques present in the assays examined was estimated from the tissue culture infective dose of 50% of the virus inoculum.

23.0-kbp HindIII A or 2.89-kbp Pst K fragments in GmFP3 were retained in the respective rescued viruses (Fig. 9).

The presence of the deletion in GmFP1 and the second insertion in GmFP3 allowed us to prove that the revertant or rescued viruses were not MP contaminants but were a result of recombination with GmMNPV HindIII-J or reversion through the loss of the inserted host sequences.

Analysis of infected-cell-specific proteins. Cultures of *S. frugiperda* cells were infected with the virus isolates at multiplicities of 10 PFU per cell and labeled at 9, 18, and 24 h postinfection for 3 h with [³H]leucine. The proteins were denatured, separated on 10% SDS-polyacrylamide gels, and detected by fluorography as detailed in Materials and Methods. A 25,000-molecular-weight (25K) infected-cell-specific protein was missing at early and late times postinfection in cells infected with each of the FP mutants, whether they were derived from GmMNPV or AcMNPV. This difference was most apparent at 18 to 21 h postinfection (Fig. 10), and it is noteworthy that the AcFP2 mutant, which had no detectable insertion or deletion in HindIII-I, also did not produce the 25K protein (Fig. 10).

An analysis of the infected-cell-specific proteins produced by GmFP1r and GmFP3r demonstrated that the expression of the 25K protein was restored on replacement of the HindIII J fragment (data not shown).

DISCUSSION

GmMNPV and AcMNPV undergo recombination with *T. ni* cell DNA at a specific site in the virus genome (AcMNPV HindIII-I) to produce mutants which contain host DNA sequences and

express the FP phenotype. In our study, FP mutants were isolated at random within three serial passages of defined parental genotypes to minimize the possibility of multiple mutations. The most common detectable mutation associated with the FP phenotype was the insertion of host sequences in the GmMNPV HindIII J or AcMNPV HindIII I fragment. The four AcMNPV FP mutants isolated by Potter and Miller (32) after 25 passages in cell culture did not have detectable insertions in HindIII-I. Their results, along with our analysis of the AcFP2 mutant, indicate that in some mutants the FP phenotype may arise without detectable insertions in HindIII-I, suggesting that other kinds of mutations in this region, or perhaps mutations at other regions in the genome, may also cause FP plaque morphology mutants.

Six of the FP mutants isolated had insertions which contained sequences homologous to *T. ni* cell DNA. Although our results were only semi-quantitative, the degree of homology to cell DNA seemed to vary among several of the insertions examined. The GmFP1 insertion sequences hybridized more intensely with the cell DNA probe than did the other FP insertions examined, whereas insertion sequences in GmFP3 hybridized very weakly with the labeled cell DNA and may therefore represent unique sequences of the *T. ni* cell genome.

The additional DNA in GmFP2 which exhibited intermediate homology to labeled cell DNA contained sequences that were dispersed and moderately repetitious in the *T. ni* cell genome. In addition, the majority of the host DNA in GmFP2 represented a distinct repeat sequence that contained a 1,280-bp *Alu*I fragment and may be considered an *Alu* family of repeats in the *T. ni* cell genome. There was no evidence to suggest that this *Alu* family bears any structural or functional similarity to those *Alu* families characterized in mammalian genomes (for a review, see reference 20), but it is interesting that repeated sequences homologous to human *Alu* sequences have recently been identified in lower eucaryotes such as slime molds and echinoderms and also in amphibians and birds (41). Homology was found between the host cell sequences in GmFP2 and large *Eco*RI, *Hind*III, *Sal*I, and *Bam*HI fragments of *T. ni* cell DNA, indicating that the repeated *Alu* sequences may be part of a larger repeat unit or that they may be clustered in the cell genome. Such clustering of repeated sequences is well documented (8, 20).

Although the GmFP2 insertion did not appear to be the most repetitious *T. ni* cell sequence which was acquired in the FP mutants we analyzed, host sequences homologous to the GmFP2 insertion were found most frequently in the FP mutant insertions. It is possible that these

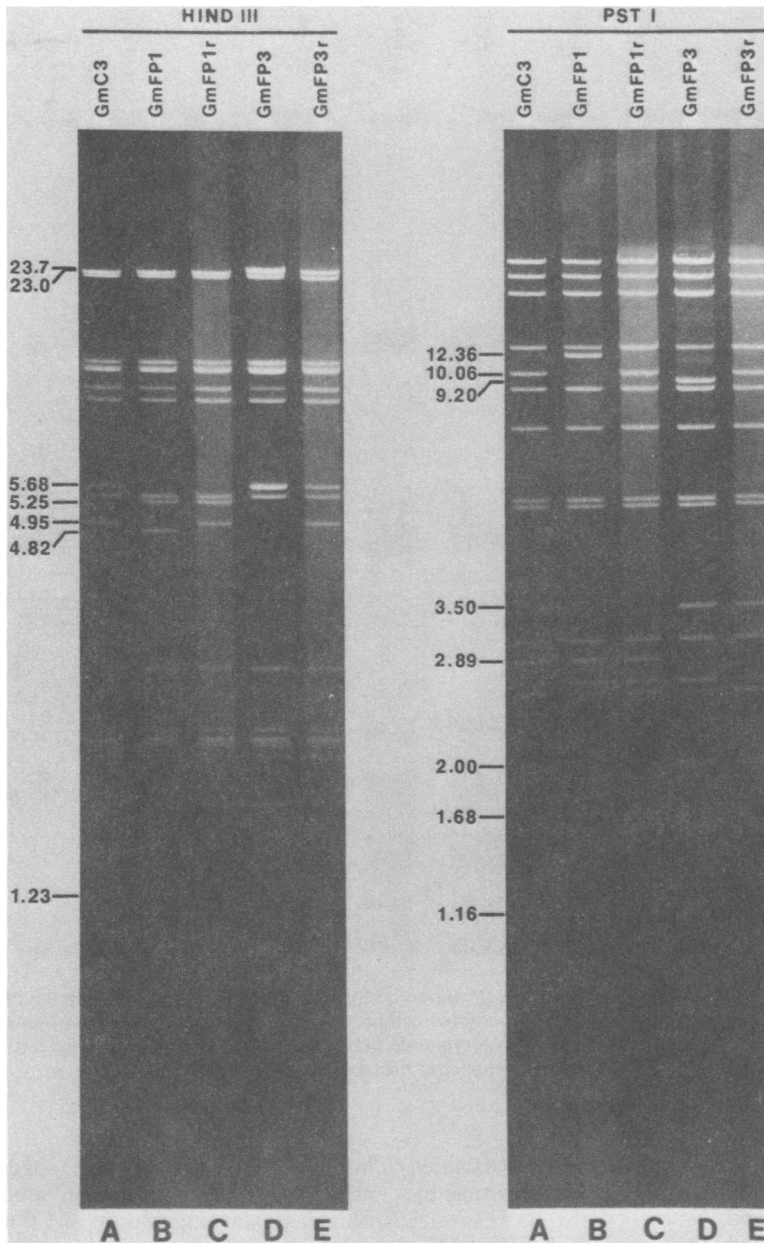


FIG. 9. Restriction enzyme analysis of FP mutants rescued with GmMNPV *Hind*III-J. Viral DNAs of GmMNPV C3 (GmC3, lane A), GmFPs 1 and 3 (lanes B and D, respectively), and GmFP1r and GmFP3r rescued FP mutants (lanes C and E, respectively) were digested with either *Hind*III (HIND III) or *Pst*I (PST I), and the fragments were electrophoresed in 0.75% agarose gels. The sizes (kbp) of certain fragments are listed to the left of each gel.

sequences were acquired more frequently because they had greater homology to viral sequences at the insertion site, or they may be moveable genetic elements.

The insertion in the AcFP6 mutant was also homologous to a class of dispersed, moderately repetitious sequences in the *T. ni* cell genome.

AcFP6 was isolated from virus which was propagated in *T. ni* larvae before inoculation of *S. frugiperda* cell cultures, which suggests that viral mutants which exhibit the FP phenotype and contain host cell DNA may also occur in infected insect larvae. This is consistent with earlier observations by Fraser and Hink (14)

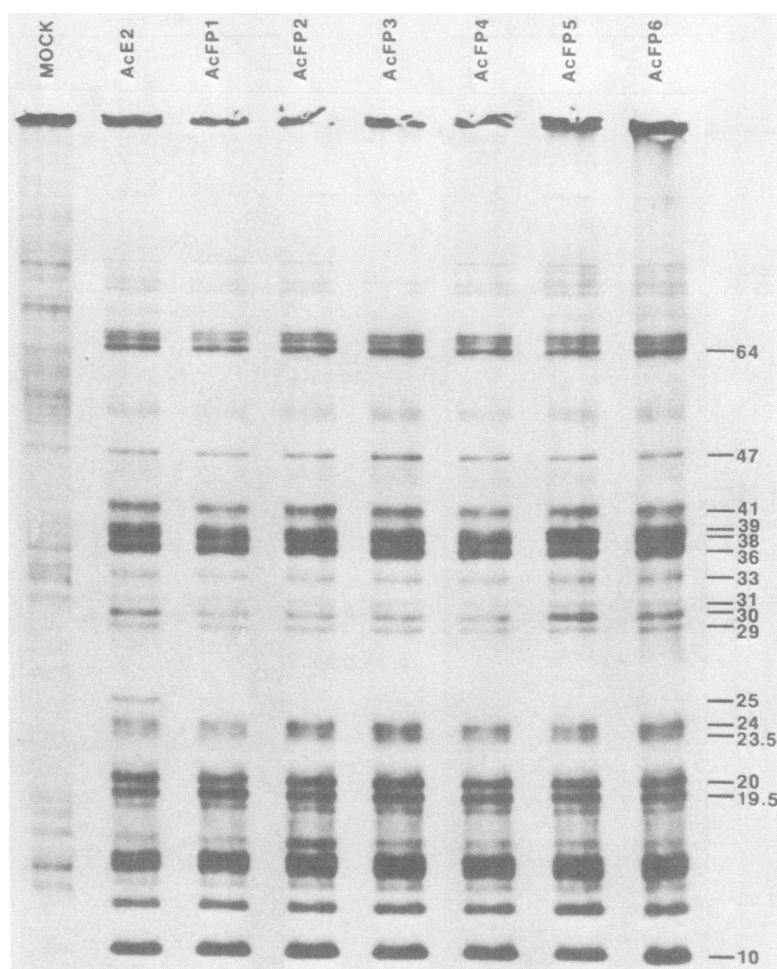


FIG. 10. Infected-cell-specific proteins. *S. frugiperda* (IPLB-SF21AE) cells were infected with AcMNPV E2 (AcE2) and AcFP mutants 1 through 6. The infected cell proteins were labeled for 3 h at 18 h postinfection with [3 H]leucine and electrophoresed in 10% polyacrylamide gels. The labeled proteins were detected by fluorography. Molecular weights ($\times 10^3$) of certain major viral proteins are indicated on the right.

which showed that plaque-purified MP isolates will produce FP mutants upon serial passage by injection of larvae.

The absence of a 25K protein in FP-infected cells was a characteristic of all FP mutants we analyzed. We have shown that replacement of the *Hind*III fragments containing cell DNA insertions in GmFP1 and GmFP3 with GmMNPV *Hind*III-J rescued the MP phenotype and restored expression of the 25K protein. There is a 25K protein which maps to a region within AcMNPV *Hind*III-J (14.7 to 18.4 map units) (43). It is possible that the 25K protein mapped previously is not the same protein that is affected in the FP mutants. The only mutant in our studies which did not have a detectable insertion in *Hind*III-I, the AcFP2 mutant, was also miss-

ing the 25K protein. This suggests that the absence of the 25K protein in infected cells may be a distinguishing feature of FP mutants.

Results from the marker rescue experiments demonstrated that most FP mutants were apparently stable but some, such as GmFP3, can spontaneously revert. These results may help explain the apparent inconsistencies in previous reports of the stability of FP mutants (14, 18, 31).

The acquisition of host DNA sequences by many animal viruses is a well documented phenomenon and was recently observed in baculoviruses (28). Miller and Miller (28) described an FP mutant which contains a 7.3-kbp host DNA insertion that structurally resembles a *D. copia*-like transposable element. The insertion se-

quences we observed in FP mutants of AcMNPV and GmMNPV were considerably smaller and were acquired at a different location in the viral genome from that reported by Miller and Miller (28), and we have no evidence that these insertions are transposable elements. In addition, adenoviruses and simian virus 40 are known to produce mutants which have acquired host DNA upon serial passage in cultured cells (11, 25, 29, 30). These mutants arise during serial passage at high multiplicities in vitro and usually exhibit a loss of viral DNA (11, 25, 29, 30). Most of these recombinational mutants are defective and dependent upon helper viruses for replication (11, 25, 29, 30). Insertion and substitution mutants which are nondefective and have acquired cellular DNA sequences at a specific region of the genome have also been isolated from adenovirus type 5 (21). Recombinational mutants of these viruses are apparently related to their ability to integrate into the cellular genome (3, 11). Unlike most simian virus 40 and adenovirus recombinational mutants, the FP mutants of baculoviruses are nondefective for viral replication (14, 31), and we have not detected a loss of viral sequences concomitant with the insertion of host sequences in FP mutants. Perhaps the FP insertion mutants, like simian virus 40 and adenovirus recombinational mutants, are related to the integration of baculovirus DNAs into their host cell genomes.

To summarize, the FP phenotype was associated with the insertion of host DNA sequences in a restricted region corresponding to the AcMNPV *Hind*III I fragment in eight of nine mutants analyzed. Furthermore, all host DNA insertions detected in this study were located within a 500-bp segment between 35.0 and 37.7 map units in the AcMNPV genome (10). The host sequences acquired by each of the FP mutant viruses were unique, suggesting that the virus directs the acquisition of host DNA to this region of the genome. Several of the host inserts had restriction sites in common and contained homologous sequences. Finally, the acquisition of host cell DNA by AcMNPV was apparently not restricted to the cell culture system but may also occur in vivo, suggesting the possible involvement of this phenomenon in the evolution of baculoviruses.

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